A strategy using isotopic fine structure to reveal potential biomarkers showing the effects of traditional Chinese medicines on Alzheimer disease in rats

Alzheimer disease (AD) is a progressive, unremitting, neurodegenerative disease characterized by progressive memory decline and subsequent loss of broader cognitive functions.

Introduction

As the pathogenesis and progression of AD remain unclear, no curative treatment is currently available to slow down or stop the degenerative effects of AD until now. *Rhodiola crenulata* has been widely served as antifatigue, antidepressant and health food for many years in China. Recently, researches showed that not only the *Rhodiola crenulata* extract (RCE) but also its major component, salidroside, has ameliorative effects on the learning and memory deficits in the treatment of AD. However, the therapeutic mechanisms underlying the protective effects of RCE against AD are still unclear. In this study, a metabolomic strategy based on accurate mass and isotopic fine structure (IFS) by Magnetic Resonance Mass Spectrometry (MRMS, traditionally known as FT-ICR MS), was established to explore the effects of *Rhodiola crenulata* extract (RCE) on Alzheimer disease (AD) in rats.

Keywords: MRMS, traditional Chinese medicines, Rhodiola crenulata, metabolomic study, accurate mass, Isotopic fine structure

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Experimental

Experimental AD model was induced in rats by bilateral hippocampal injection of $A\beta_{1\text{-}42}$, and Morris water maze task (MWM) was used to evaluate the effects of RCE on AD. The metabolomic study was performed using a solariX 7T MRMS system. The experimental workflow consisted of HPLC-MRMS, fraction collection and direct infusion (DI)-MRMS to screen and identify the potential biomarkers, Figure 1. Elemental compositions were determined using the following seven steps.

Step 1. Identify the monoisotopic peak, A+1, A+2 and A+3 isotopic peaks in the experimental data.

Step 2. Acquire the experimental IFS with the relative intensities of each isotopic peak.

Step 3. Assign the M+1 isotopic peaks originating from ^{15}N, ^{33}S and ^{13}C by calculating the mass difference between the monoisotopic mass and each peak and then estimate the carbon numbers based on the relative intensity of ^{13}C isotopic peak.

Step 4. Exclude enough candidates by relative intensities of ^{13}C isotopic peaks.

Step 5. Assign the A+2 isotopic peaks originating from $^{15}N^{13}C$, ^{18}O, ^{34}S and $^{13}C_2$ substitution and the A+3 isotopic peaks (if necessary) by calculating the mass difference between the monoisotopic mass and each peak.

Step 6. Acquire the theoretical IFS for candidate formulae and the relative intensities of isotopic peaks by Compass Isotope pattern Software.

Step 7. Determine the elemental composition by comparing the experimental and the theoretical data and assign a definite formula to each potential biomarker.

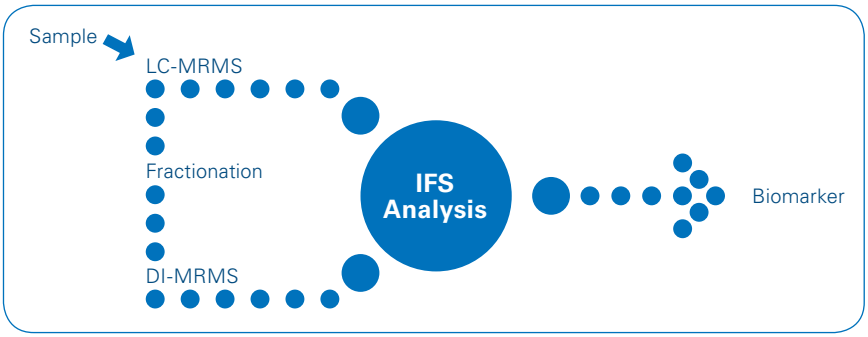

Figure 1: Workflow for identification of biomarkers based on accurate mass and isotopic fine structures by dual mode combined-MRMS.

Results and Discussion

The initial HPLC profiling results were screened by statistical differentiation to reveal potential biomarkers with an experimental mass accuracy of less than 1 ppm. The samples are then fractionated, and the fractions of interest are remeasured using ultra-high resolution MRMS to reveal the experimental IFS. An example is shown for an unknown with a retention time of 16.24 minutes and *m/z* 524.37054. Using a mass accuracy of less than 1 ppm, there are 4 possible chemical formula, shown in Table 1. The proposed formulae contain 5 elements that have stable heavy isotopes: ^{13}C, ^{2}H, ^{15}N, ^{18}O and ^{34}S. The absence of the ^{34}S isotope clearly eliminates 2 candidates. The difference in the relative intensity of the experimental heavy isotopes to the simulated pattern is used to determine the correct formula. After IFS analysis, only one candidate remained, $C_{26}H_{55}NO_7P^+$. The simulated IFS mass spectrum of $C_{26}H_{55}NO_7P^+$ at RP=1,000,000 is given in Figure 2.

A total of 20 metabolites contributing to AD progress were decisively identified, and 17 metabolites of them were restored to the control-like levels after RCE treatment (daily dose: 2.24 g/kg), shown in Table 2. The metabolic pathway analysis revealed that the disturbed pathways including tryptophan metabolism, sphingolipid metabolism and glycerophospholipid metabolism in AD model rats were regulated after high dose RCE application. It is the first time that the dual mode combined MRMS based metabolomic strategy was applied to biochemically profile the serum metabolic pathways of AD rats affected by RCE. These outcomes provide reliable evidence to illuminate the biochemical mechanisms of AD and facilitate investigation of the therapeutic benefits of RCE in AD treatment. Notably, it indicated that the developed method based on accurate mass and IFS has sufficient performance for decisive identification of biomarkers in metabolomic studies.

Table 1: Candidate molecular formula annotations for the unknown at m/z 524.37054 and retention time 16.24 minutes.

Proposed Annotation	Major IFS Peaks
$C_{26}H_{55}NO_7P^+$	$^{13}C_{26}\ ^{15}N_1\ ^{18}O_7$
$C_{29}H_{46}N_7O_2^+$	$^{13}C_{29}\ ^{15}N_7\ ^{18}O_2$
$C_{29}H_{54}N_3OS_2^+$	$^{13}C_{29}\ ^{15}N_3\ ^{18}O_1\ ^{34}S_2$
$C_{21}H_{50}N_9O_4S^+$	$^{13}C_{21}\ ^{15}N_9\ ^{18}O_4\ ^{34}S_1$

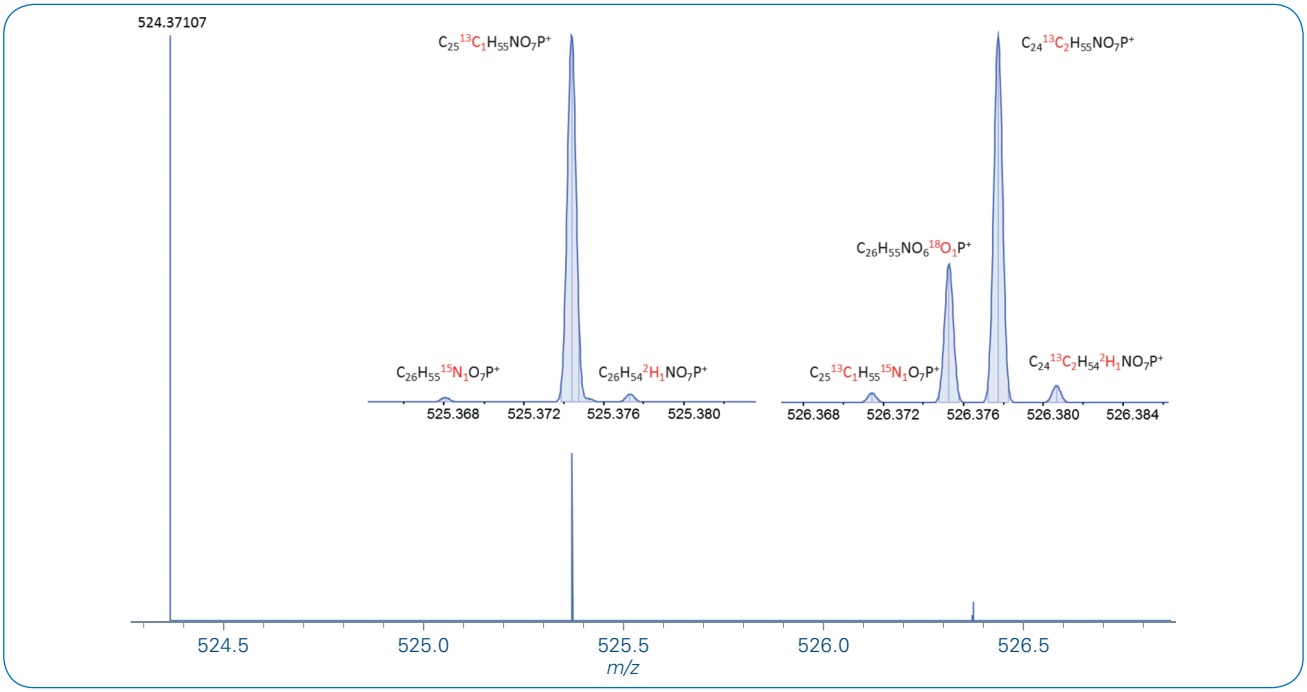

Figure 2: Simulated IFS mass spectrum for $C_{26}H_{55}NO_7P^+$ at a resolution of 1,000,000 for the monoisotopic mass.

Table 2: IFS Annotated Metabolites

Molecular Formula	Proposed Compound	Reversed
$C_{10}H_{19}NO_4$	Propionylcarnitine	Y
$C_{11}H_{21}NO4$	Butyrylcarnitine	Y
$C_{11}H_{12}N_2O_2$	L-Tryptophan	Y
$C_{43}H_{83}O_{13}P$	PI (34:0)	--
$C_{18}H_{39}NO_2$	Sphinganine	Y
$C_{24}H_{48}NO_7P$	LysoPC (16:1(9Z))	Y
$C_{26}H_{48}NO_7P$	LysoPC (18:3)	Y
$C_{26}H_{50}NO_7P$	LysoPC (18:2(9Z,12Z))	Y
$C_{24}H_{50}NO_7P$	LysoPC (16:0)	Y
$C_{28}H_{50}NO_7P$	LysoPC (20:4)	Y
$C_{23}H_{45}NO_4$	L-Palmitoylcarnitine	--
$C_{25}H_{45}NO_4$	Linoleyl carnitine	Y
$C_{30}H_{50}NO_7P$	LysoPC (22:6(4Z,7Z,10Z, 13Z,16Z,19Z))	Y
$C_{26}H_{52}NO_7P$	LysoPC (18:1)	Y
$C_{25}H_{52}NO_7P$	LysoPC (17:0)	Y
$C_{28}H_{54}NO_7P$	LysoPC (20:2(11Z,14Z))	Y
$C_{23}H_{48}NO_7P$	LysoPC (15:0)	Y
$C_{28}H_{52}NO_7P$	LysoPC (20:3)	Y
$C_{26}H_{54}NO_7P$	LysoPC (18:0)	Y
$C_{18}H_{30}O_2$	Alpha-Linolenic acid	--

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This Application Note is a condensed and revised version of the Journal of Pharmaceutical and Biomedical Analysis article "A metabolomic study based on accurate mass and isotopic fine structures by dual mode combined-FT-ICR-MS to explore the effects of *Rhodiola crenulata* extract on Alzheimer disease in rats".

References

[1] Zhang Xiaoxue, Jiang Xiwei, Wang Xue, et al. (2019) *A metabolomic study based on accurate mass and isotopic fine structures by dual mode combined-FT-ICR-MS to explore the effects of* Rhodiola crenulata *extract on Alzheimer disease in rats.* J Pharm Biomed Anal, **166**: 347-356.

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